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Comparative Histological Analysis of Tongue Structure in adult Local and Shirazi Cats

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Article Informations

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local cat, Sherazi cat , tongue, keratinization, papillae.

A B S T R A C T

The histological analysis of both the local and Sherazi cat was conducted to comprehend their distinct differences. These felines, classified under the scientific name (*Felis catus*), are members of the Felidae family . Thirty tongue samples were gathered from cats that frequented veterinary clinics, with each type of cat comprising fifteen samples. The results of our histological study of the parts of tongue in the local and adult Sherazi cat showed that it consists of three main layers: the mucosal layer, submucosa layer, and muscular layer in all its regions. The mucosal layer in the dorsal surface of the tongue is composed of keratinized stratified squamous epithelium, the tunica submucosa consisted of loose Connective tissue containing lingual glands in root region only, which are of two types: mucous and serous glands. The tunica muscularis appears as a bundles of muscle fibers in three directions, longitudinal, transverse and perpendicular. The study also found the presence of four types of lingual papillae on the dorsal surface of the tongue (filiform, fungiform, foliate, and circumvallate papillae).

Introduction

The family Felidae constitutes a subgroup in the animal kingdom characterized by its high specialization as predators. It consists of two main subspecies, the tiger and the cat (*Felis catus*). Felids are carnivorous mammals with advanced physical features that allow them to adapt to a hunting lifestyle (Bateman & Fleming, 2012). Felidae have notable morphological similarities between species, as they all include a distinctive skull designed for hunting prey and crushing bones. Among this family come cats, which are the only domesticated animal. Comparative studies of living organisms are considered one of the most important scientific foundations for understanding biological and evolutionary diversity and physical structure. Among these creatures are cats, as the tongue of cats is considered an important part of the physical structure, which performs multiple functions related to tasting, communicating with each other, eating food, and reducing body temperature. The tongue exists as a structure, muscular within the oral cavity, connected to the lower jaw via the lingual frenulum (HUSSEIN & IBRAHIM, 2023), (Eubanks, 2007).The cat's tongue consists structurally of multiple groups of cells and tissues that perform its various functions, which represent epithelial tissue, muscles, blood vessels, nerve fibers, and various types of glands, in addition to groups of specialized structures found in the some lingual papillae on the surface of the tongue and considered the main part in determining different tastes, called taste buds (HUSSEIN & IBRAHIM, 2023). The objectives of the study include analyzing the general structure of the tongue and accurately describing the internal tissue structure, in addition to describing the papillae in the domestic cat and comparing it with the Sherazi cat. This comparative analysis will contribute to drawing a comprehensive picture of the histological similarities and differences between the two breeds.

Materials and Methods:

The study design was to conduct 15 local cats and the same number of Sherazi cats, using histological study by light microscope. Tissues will be carefully analyzed, starting with the histological description of the tongue, and proceeding to the description of tissue structures such as the mucous membrane, glands, and internal structures. The types of histological papillae will also be identified, which include filiform, fungal, foliate, and circumvallate papillae. Different types of stains will be used, including Harris hematoxylin and eosin (H&E), Masson's trichrome, and PAS-$AB_{ph2.5}$.

1. A variety of devices and chemicals were used in the study. Among the devices used were an ELECTRO_MAG m420 BP oven, an Electro thermal BG 7311 water bath, and a WES WOX optic model 1090A rotary microtome. In addition, use an Olympus Dm-CBAD light microscope, a USB 2.0 scope image cam, a Lab-smith hot plate, and a sensitive balance from EK - I -EW - I. As for the chemicals used, they included distilled water, 10% formalin, absolute ethyl alcohol, xylene, paraffin wax with a melting point of 58%, and Harris' hematoxylin and eosin stain. (Harris hematoxylin stain and eosin), Masson's trichrome stain, Alcian blue (2.5) periodic acid_shiff, and Bouin's solution. And glacial acetic acid(Layton et al., 2019).

2.Sample collection:
(30) cats were collected in this study, and they were divided equally between two groups: a local adult cat group (15 samples) and adult Sherazi cat group (15 samples), without regard to gender. The animals of the study include cats that had been involved in accidents and whose recovery was challenging (Terminally ill animals), and that were brought to veterinary clinics, as shown in Figures (1) and (2).All experimental animals were set in study after approvment of scientific commette/Coll.vet.Med/ university of Mosul (ref. No:UM.VET.2023.084 ; Issue date:15/8/2023)

Figure 1: Sherazi cats.

Figure 2: Local cats.

Each study animal is weighed before being given a high dose of anesthetics to end its life (euthanasia). After the animal dies, the tongue is extracted by dissecting the oral cavity.

Histological study:
1 - For the purpose of conducting a histological study and investigating the structures of the tongue more precisely, the tongue in both animals was divided longitudinally from the apex to the root into two symmetrical parts (a right part and a left part),

and then the two longitudinal parts were sub subdivided transversely into (7) transverse parts, as shown in Figure (3). (4).

2- 1cm² sized samples were taken from all pieces of the tongue and fixed in a 10% neutral buffer formalin solution for (48-72) hours(Culling, 1985; Luna, 1968).

3- The samples were passed with increasing concentrations of ethyl alcohol from 70%-100% for the purpose of dehydration.

4- The models were passed with Xylen for (15 minutes) for the purpose of clearing.

5- The samples were passed in paraffin wax at a temperature of 58-60 degrees Celsius (3 passes) for the purpose of impregnation (Bancroft et al., 2019).

6- Then the models were embedded in paraffin wax to make wax molds. (Culling, 1985; Luna, 1968)

7- The wax blocks were cut using a rotary microtome to obtain tissue slices with a thickness of 5-6 micrometers.

8- Use the following stains to study different tissue structures:

A- Harris Hematoxylin and Eosin stain to study the general histological structure of the tongue.

B- Masson's Trichrome stain to differentiate between muscle fibers and collagen fibers. (Luna, 1968; Layton et al., 2019)

C- Alcian blue pH 2.5 combined with Periodic acid Schiff (ABph2.5-PAS) to detect the type of mucin in the tongue glands (Layton et al., 2019).

The tissue slides were photographed using a digital camera (USB 2.0 scope image) attached to an optical microscope (Olympus light microscope).

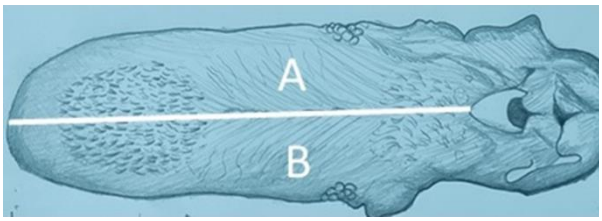

Figure 3: (A) shows the right part of the tongue, (B) shows the left part of the tongue in both breeds.

Figure 4: (1) A first section of the free part of the tongue , (2) A second section of the free part of the tongue, (3) A third section of the free part of the tongue, (4) A first section of the body of the tongue, (5) A second section of the body of the tongue, (6) A third section of the body of the tongue, (7) the root of the tongue.

Statistical analysis:

Means and standard errors were calculated for the histological measurements in the current study for both tongue of local and Sherazi adult cats using the software (IBM Spss V25, UK). The presence of statistically significant differences was confirmed using the T-test for independent samples at a significant value of $p \leq 0.05$ (Petrie and Watson, 2013).

Result:

Description of the mucous membrane surrounding the tongue:

The results of our histological study of the parts of the tongue in the local cat and the adult Sherazi cat showed that it consists of three main layers: the mucosal layer, the submucosal layer, and the muscular layer in all its regions, as shows in Figure (5).

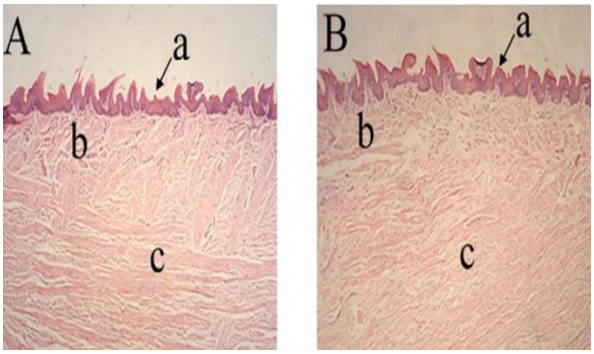

Figure 5: Microscopic section in the tongue of local cat (A) and
a Sherazi cat (B) showing the mucosal layer (a), submucosal layer (b),and muscular layer (c), (H&E) stain , (X400).

The mucous layer on the dorsal surface of the tongue consists of keratinized stratified squamous epithelium. The keratinized epithelium consists of four layers:

1. The basal layer (stratum basale). 2. Stratum spinosum. 3. Stratum granulosum. 4. Stratum corneum. As in Figure (6).

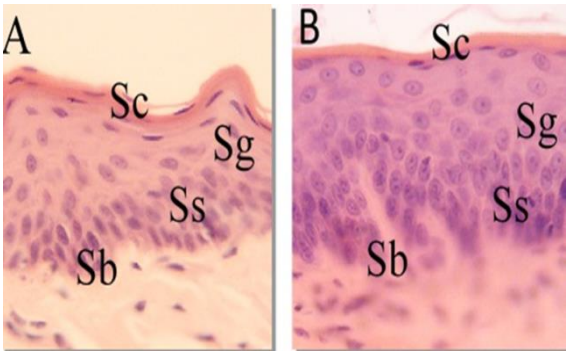

Figure 6: Microscopic tissue section of the tongue in local cat (A) and Sherazi cat (B). Stratum basale

(Sb), stratum spinosum (Ss), stratum granulosum (Sg), stratum corneum (Sc), (H&E) stain, (X400).

Our study showed differences in the thickness of the stratum corneum in parts of the tongue in cats, both within a single species or between local and Sherazi species.
Difference were also noticed in thickness between the edge and middle in the same part of the tongue, with significant differences between the two types. Also, the thickness of the epithelium and the lamina propria varied between the Periphery and the middle in the parts of the tongue, with
significant differences between local cats and Sherazi cats, with the exception of the third free part of the tongue, where did not notice a significant difference between the two types. As in the table(1) .

Our study revealed that the stratum corneum in the tongue of the local cat is thickest at the Periphery of the parts, and decreases toward the center. We also found that this pattern is similar between the local cat and the Sherazi cat, but there were differences in the values between them, indicating that there were significant differences between them. It was also shown that the root area of the tongue is free of keratinization in both species. Regarding the thickness of the epithelium of the tongue body, variations were found between the parts of the tongue of each animal and between species. For example, the greatest thickness of the stratum corneum in the local cat was at the middle third part of the body, while the smallest thickness was located at the ends of the three parts of the tongue. As for the Sherazi cat, the greatest thickness of the stratum corneum was located at the ends of the three parts of the tongue, while The lowest thickness was located in the middle third of the body. The results show that there are significant differences in the thickness of the stratum corneum between the local cat and the Sherazi, with the Sherazi being superior in some parts. With regard to the lamina propria, its thickness varies between parts and between the two species, with significant differences between them, and different trends in it. We also noticed that there is a difference in the thickness of the lamina propria at the root of the tongue between the two types, without any significant differences between them. As in Table (2).

In addition, our study showed that the thickness of the mucous membrane surrounding the tongue differs between the dorsal surface and the ventral surface of the tongue in both types of local and Sherazi cats. It was noted that the dorsal surface consists of keratinized stratified squamous epithelium and contains structures called lingual papillae. The epithelium of the ventral surface of the tongue is non-keratinized stratified squamous epithelium and does not contain lingual papillae as in Figure (7).

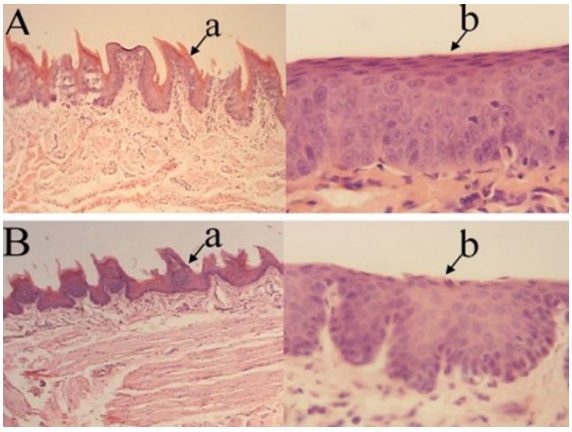

Figure 7: Microscopic tissue section of the tongue of the local cat (A) and the sherazi cat (B), the dorsal surface of the tongue in both animals (a), the ventral surface in both animals (b), (H&E) stain , (X400).

It was also observed that the muscle layer consists of skeletal muscles with longitudinal, vertical and transverse directions, which form right angles to each other as in Figure (8).

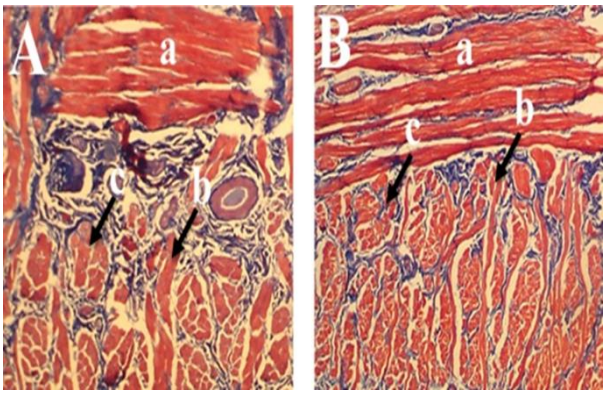

Figure 8: Microscopic tissue section of the tongue of the local cat (A) and the Sherazi cat (B), longitudinal muscle (a), vertical muscle (b), transverse muscle (c) in both animals, (Masson's Trichrome Stain) (X100).

Our histological study of all areas of the tongue and in both types of cats showed the absence of any type of gland in it except for the third part close to the root and the root of the tongue, as these two parts contain multiple secretory lingual salivary glands, some of which have mucous secretions, or serous and mixed secretions in other sections. The type of secretion was distinguished by means of special pigments and the shape of the cells in the secretory units. The glands with mucous secretions were distinguished by their low columnar cells and their relatively wide lumen, and the nuclei of the cells were dark basal in color in Bass Alchian blue stain, while the glands with serous secretion were characterized by their cells being

pyramidal in shape. With a nucleus located in the center of the cell, spherical in shape. As in Figure (9)

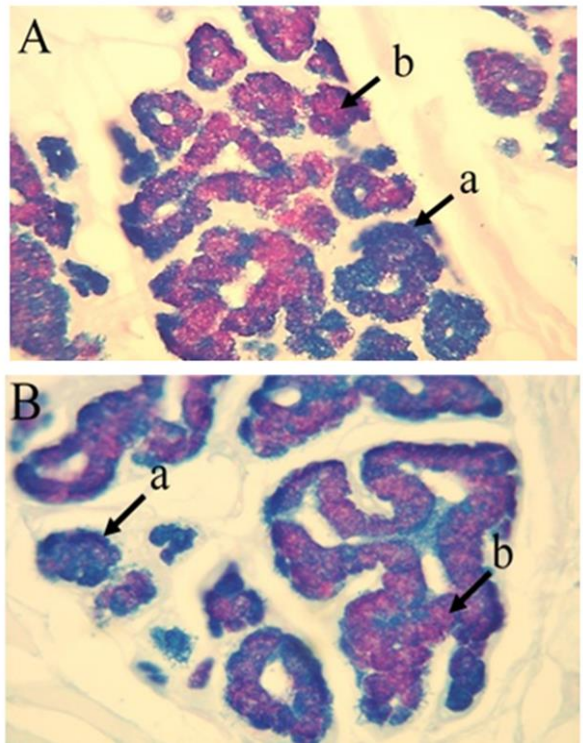

Figure 9: A microscopic tissue section showing the types of glands (mucous, serous, mixed) in the tongue of the local cat (A) and the Sherazi cat (B), mucous glands in both animals (a), serous glands in both animals (b), ABph2.5-PAS (X400).

Discussion:

The results of our histological study of parts of the tongue in the local cat and the adult Sherazi cat showed that it consists of three main layers: the mucosal layer, the submucosa layer, and the muscular layer. Thus, we agree with (HUSSEIN & IBRAHIM, 2023) in the study conducted on the tongue. The adult Iraqi domestic cat (Felis catus), and (Al-Jumaily *et al*., 2021) in the tongue of the ferret, where they mentioned that the tongue is composed of three main layers (mucous layer, submucosal layer, muscular layer), and it seems that this The difference is related to the structural composition based on the functional requirements.

Our study showed variation in the thickness of the stratum corneum of the tongue in different species of cats, including differences between local and Sherazi cat species, in addition to differences within a single cat species. This discrepancy was observed in the same part of the tongue, where the difference between the edge and the middle of the part could be observed. This variation is due to different evolutionary adaptations and genetic diversity between different species of cats. local cats may have evolved differently to adapt to their environment and lifestyle compared to imported or artificially bred cats such as the Sherazi. As for the difference in the same part of the tongue, it may reflect genetic diversity within different species and the complex influences that play a role in shaping the development of organs and vital systems of living organisms.

Our current study has shown that the thickness of the mucous membrane surrounding the tongue varies between the dorsal surface and the ventral surface of the tongue in both types of domestic and Persian cats. It has been noted that the dorsal surface consists of Keratinized stratified squamous epithelium and contains structures called lingual papillae because it is exposed to friction. The erosion is greater due to the presence of food and liquids that pass through the mouth, while the epithelium of the ventral surface of the tongue is non-keratinized stratified squamous epithelium and does not contain lingual papillae because it is more susceptible to moisture and contact with moist tissues inside the mouth. With these results, we agree with researchers (AL-Mahmodi, 2016) in the rabbit's tongue, and (Sadeghinezhad et al., 2017) in the tongue of the Persian tiger (Panthera pardus saxicolor), where they mentioned in their study that the dorsal surface of the tongue is a keratinized stratified squamous epithelium. The ventral surface of the tongue is not keratinized.

Our histological study of the cat tongue revealed the presence of lingual salivary glands only in the third segment and the root of the tongue. These glands are characterized by multiple secretions of saliva, including mucous and serous saliva. The secretions are distinguished by special stains and cell shape. The cells in the mucous glands differ from the serous glands in their shape and the distribution of their nuclei due to their different functions. The former specializes in secreting mucus for lubrication and protection, while the latter specializes in secreting digestive enzymes and proteins for the digestion process. With these results we agree with (HUSSEIN & IBRAHIM, 2023) in the study. Which was conducted on the tongue of the adult Iraqi domestic cat (Felis catus), and (Salih et al., 2012) on the tongue of the rabbit, and (Ibrahim & Al-Jumaily, 2020) on the tongue of the ferret (Herpestes javanicus), and we disagree with (Cizek *et al* .,2012) in the tongue of the lesser hedgehog (Echinops telfairi), where it is located in the muscle layer. The reason for this difference may be attributed to functional requirements and their relationship to different nutritional patterns in the animal.

This study showed that the presence of different shaped structures known as lingual papillae on the dorsal and lateral surface of the tongue in local cats and Sherazi cats, including mechanical and chemical papillae. Mechanical papillae do not have taste buds and perform a mechanical function such as pushing food and drinking water, while chemical papillae contain taste buds and play a role in taste. No type

of papillae was found on the ventral surface of the tongue in cats. These results differ from (Emura *et al*., 2000) in the tongue of the jungle dog (Speothos venaticus), where they found three main types of papillae: filiform, fungal, and circumvallate papillae, and absence of the foliate papillae.

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Competing Interests

The authors declared that there is no conflict of interest.

References:

[1] Kitchener, A. C., Breitenmoser-Würsten, C., Eizirik, E., Gentry, A., Werdelin, L., Wilting, A., ... & Tobe, S. (2017). A revised taxonomy of the Felidae: The final report of the Cat Classification Task Force of the IUCN Cat Specialist Group. *Cat News*. https://repository.si.edu/handle/10088/32616

[2] Bateman, P. W., & Fleming, P. A. (2012). Big city life: carnivores in urban environments. *Journal of zoology*, *287*(1), 1-23. https://doi.org/10.1111/j.1469-7998.2011.00887.x

[3] HUSSEIN, A. A., & IBRAHIM, M. K. (2023). Histological study of lingual papillae on the tongue of the adult Iraqi domestic cat, Felis catus. Iranian Journal of Ichthyology, 10, 105-111. https://ijichthyol.org/index.php/iji/article/view/923

[4] Eubanks, D. L. (2007). Anatomy and clinical examination of the tongue in the dog. *Journal of Veterinary Dentistry*, *24*(4), 271-273. https://doi.org/10.1177/089875640702400413

[5] Layton, C., Bancroft, J. D., & Suvarna, S. K. (2019). Fixation of tissues. Bancroft's Theory and Practice of Histological Techniques (Eighth Edition): Content Repository Only, 40-63. https://shop.elsevier.com/books/bancrofts-theory-and-practice-of-histological-techniques/suvarna/978-0-7020-6864-5

[6] Culling , C. F. A. ; Allison , R. T.and ; Barr , W. T. (1985) . Celluler pathology technique 4th ed. Butterworth., pp:16,167,214,215,216. https://shop.elsevier.com/books/cellular-pathology-technique/culling/978-0-407-72903-2

[7] Luna LG. (1968). Manual of histologic staining methods of the armed forces institute of pathology 3rd (ed.): Mc Graw-Hill book Co. N.Y. PP: 32-153. https://pesquisa.bvsalud.org/portal/resource/pt/biblio-1081120

[8] Layton, C., Bancroft, J. D., & Suvarna, S. K. (2019). Fixation of tissues. Bancroft's Theory and Practice of Histological Techniques (Eighth Edition): Content Repository Only, 40-63. https://shop.elsevier.com/books/bancrofts-theory-and-practice-of-histological-techniques/suvarna/978-0-7020-6864-5

[9] Petrie, A. and Watson, P. (2006). Statistics for Veterinary and Animal Science 3E. 3rd edn. oxford UK: Wiley-Blackwell. https://www.google.iq/books/edition/Statistics_for_Veterinary_and_Animal_Sci/EoJCMAEACAAJ?hl=ar

[10] Al-Jumaily, I. S. A., Ibrahim, M. K., & Abid, W. B. (2021, May). Histological structure of the Tongue in Mongoose (Herpestes javanicus). In Journal of Physics: Conference Series (Vol. 1879, No. 2, p. 022031). IOP Publishing. https://iopscience.iop.org/article/10.1088/1742-6596/1879/2/022031/meta#:~:text=DOI%2010.1088/1742%2D6596/1879/2/022031

[11] AL-Mahmodi, A. M. M. (2016). Anatomical and Histological study of the Tongue of wild adult male Rabbits (Oryctolagus cuniculus f. domestica) in AL-Najaf province. Kufa Journal For Veterinary Medical Sciences, 7(2). https://doi.org/10.36326/kjvs/2016/v7i24341

[12] Sadeghinezhad, J., Sheibani, M. T., Memarian, I., & Chiocchetti, R. (2017). Morphological study of the Persian leopard (Panthera pardus saxicolor) tongue. *Anatomia,histologia,embryologia*, *46*(3),240-248. https://doi.org/10.1111/ahe.12263

[13] Salih, Q. M., Ahmad, N. S., & Mahmood, S. K. H. (2012). Morphological and histological studies of the tongue and lingual papillae of the local rabbit. *Basrah Journal of Veterinary Research*, *11*, 4. https://www.researchgate.net/publication/325324493_Morphological_and_histological_studies_of_the_tongue_and_lingual_papillae_of_the_local_rabbit

[14] Ibrahim, M. K., & Al-Jumaily, I. S. (2020). Morphological study of the tongue in mongoose (Herpestes javanicus). Biochemical and Cellular Archives, 20(2), 5923-5926. https://www.cabidigitallibrary.org/doi/full/10.5555/20219999331

[15] Cizek, P., Hamouzova, P., Goździewska-Harłajczuk, K., Klećkowska-Nawrot, J., & Kvapil, P. (2020). Microscopic structure of the tongue in the lesser hedgehog tenrec (Echinops telfairi, Afrosoricida) and its relation to phylogenesis. *Anatomical Science International*, *95*, 313-322. https://doi.org/10.1007/s12565-019-00522-1

[16] EMURA, S., TAMADA, A., HAYAKAWA, D., CHEN, H., & SHOUMURA, S. (2000). Morphology of the dorsal lingual papillae in the bush dog (Speothos venaticus). OkajimasfoliaanatomicaJaponica,77(5),137-141. https://doi.org/10.2535/ofaj1936.77.5_137

Table 1: Shows measurements of the thickness of the stratum corneum, the thickness of the epithelium, and the lamina propria with a micrometers (µm) in the tongue parts of local and Sherazi adult cats.

Type	Variables	Free Part 1 (M ± SEM)	Free Part Middle 1 (M ± SEM)	Free Part 2 (M ± SEM)	Free Part Middle 2 (M ± SEM)	Free Part 3 (M ± SEM)	Free Part Middle 3 (M ± SEM)
Local	Thickness of the keratinized segment (µm)	0.55±0.38	0.48±0.02*	0.32±0.01	0.45±0.01*	0.40±0.001	0.25±0.01
	Epithelium thickness (µm)	6.71±0.10	5.70±0.20	3.28±0.33	4.31±0.29	5.96±0.17	0.25±0.01
	Thickness of lamina propria (µm)	55.44±1.59*	22.49±1.20*	24.56±0.50	20.29±1.49*	20.70±0.66	17.28±0.83*
Sherazi	Thickness of the keratinized segment (µm)	0.51±0.02	0.38±0.03	0.44±0.01*	0.21±0.01	0.40±0.001	0.21±0.01
	Epithelium thickness (µm)	6.20±0.12	5.82±0.11	6.98±0.17	5.65±0.44	5.96±0.17	5.60±0.44*
	Thickness of lamina propria (µm)	24.82±1.93	8.71±0.40	20.70±0.55	7.64±0.26	19.70±1.24	7.58±0.25

M±SEM: mean and standard error

*: There are statistically significant differences at the level of significance (P≤ 0.05) between the local cat and the adult Sherazi cat

Table 2. Shows measurements of the thickness of the stratum corneum, the thickness of the epithelium, and the lamina propria with a micrometer (µm) in the body of the tongue of the local cat and the adult Sherazi cat.

Type	**Variables**	**Body 1 M±SEM**	**Medium body 1 M±SEM**	**Body 2 M±SEM**	**Medium body 2 M±SEM**	**Body 3 M±SEM**	**Medium body 3 M±SEM**	**root M±SEM**	**Middle root M±SEM**
Local	**Thickness of keratinized part (µm))**	0.25±0.01*	0.16±0.01	0.25±0.01*	0.14±0.01	0.25±0.01*	0.13±0.01	X	X
	Epithelium thickness (µm)	3.28±0.15	3.83±0.6	3.28±0.15	3.78±0.06	3.28±0.15	4.18±0.15	2.30±0.04	2.31±0.03
	Thickness of lamina propria (µm)	6.15±0.18*	4.95±0.33	6.15±0.18*	4.63±0.22	6.15±0.18*	4.44±0.19	13.38±0.86	12.78±1.23
Sherazi	**Thickness of the keratinized segment (µm)**	0.17±0.01	0.13±0.03	0.17±0.01	0.12±0.02	0.17±0.01	0.12±0.02	X	X
	Epithelium thickness (µm)	5.30±0.34	4.34±0.19	5.30±0.34	4.21±0.15	5.30±0.34	4.18±0.15	2.77±0.16	6.20±0.10
	Thickness of submucosal layer (µm)	7.58±0.25	8.12±0.53*	7.58±0.25	8.34±0.51*	7.58±0.25	8.36±0.49*	11.66±1.41	13.14±0.96

M±SEM: mean and standard error

*: There are statistically significant differences at the level of significance (P≤ 0.05) between the local cat and the adult Sherazi cat.